2712-Pos Board B142
Live-Cell Single-Molecule Imaging of Sense and Antisense Transcription of a Yeast Gene
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The eukaryotic genome is pervasively transcribed, giving rise to various sorts of non-coding RNAs whose mechanisms of action are for the most part not understood. Recent technological advances now allow direct visualization of the synthesis of nascent transcripts from individual genes over time by decorating RNAs with fluorescent proteins. Using the orthogonal RNA-binding MS2 and PP7 bacteriophage coat proteins, we were recently able to tag two regions of the same RNA in two different colors [Coulon et al. 2014, eLife, in press]. Here, we used this technique to visualize simultaneously sense and antisense transcription from the GAL10 locus in yeast, during activation of the GAL pathway. Fluorescence fluctuations recorded in both channels at the transcription site reflect the kinetics of transcription on both strands as the GAL10 gene gets activated in response to galactose. We observe transient antisense transcription occurring almost exclusively prior to the appearance of sense transcription. Using cross-correlation analysis, we uncovered specific temporal windows relatively to sense activation where antisense transcription is enriched or depleted - likely reflecting the biochemical mechanisms underlying activation. Once transcription of the GAL10 gene starts, transcripts are produced in bursts separated by periods of inactivity, occasionally leaving the opportunity for antisense transcription to happen. We developed a method for applying fluctuation correlation analysis to non-stationary time traces. This allowed us to isolate the bursting kinetics even in the non-steadystate context of a transient response to galactose. By modeling the autocorrelation of a bursting gene, we were able to infer from our data how the elongation rate, burst size and burst frequency of the GAL10 gene are modulated by different doses of galactose. This work shows how in vivo single-molecule methods and fluctuation analysis can reveal unanticipated mechanisms of transcriptional regulation.

2713-Pos Board B143
The Mechanism of Transcription Stalling under Torsion
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During transcription, RNA polymerase (RNAP) translocates along DNA and introduces torsional stress, and excessive torque accumulation can lead to transcription stalling. We previously measured this stall torque for *E. coli* RNAP during transcription[1]. However the mechanism and regulation of RNAP stalling under torsion has remained elusive. Here, we investigate the transcriptional dynamics of RNAP in the presence and absence of GreB, a transcription elongation factor known to rescue backtracked RNAP. Using an angular optical trap assay, we found that the presence of GreB can significantly increase the stall torque of RNAP. In addition, RNAP often exhibited distinct reverse motion upon stalling in the absence of GreB, while this was rarely observed in the presence of GreB. These results suggest that backtracking is the primary mechanism of RNAP stalling on DNA under torsion and demonstrate a potential regulatory role for GreB in assisting an elongating RNAP in overcoming the torsional barrier of DNA.
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2714-Pos Board B144
A High-Throughput Single-Molecule Assay for Screening Transcriptional Interference
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Transcription of genetic information from DNA to RNA for protein synthesis and regulatory control is a fundamental biological process. Gene expression in the cell is regulated, and eventually silenced, for various purposes such as to trigger a developmental pathway, to protect the genome from infectious DNA elements or to respond to environmental stimuli. Gene silencing techniques are used in biomedical research and in the development of novel therapeutics for treating various cancers, infectious diseases and neurodegenerative disorders.
We present a simple single-molecule assay for studying transcription and assessing the effects of small molecules on gene transcription. Our approach combines aspects of tethered particle motion (TPM) microscopy with total internal reflection fluorescence (TIRF) microscopy. This method allows us to watch RNA transcription occurring on dozens of single DNA molecules in parallel and may be scaled for use in higher-throughput investigations of transcriptional activity.
To illustrate the utility of our method, we have been investigating transcriptional interference caused by the histone-like nucleosomal protein (H-NS), which is a global transcriptional silencer abundant in many bacteria. Here we use this technique to explore the promoter occupancy and procession of T7 polymerase in the presence of H-NS. However, our setup is amenable to the study of a host of different regulatory factors acting on a variety of promoter architectures.

2715-Pos Board B145
DNA Looping both Enhances and Suppreses Transcriptional Noise
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DNA looping has been observed to enhance and suppress transcriptional noise but it is uncertain which of these two opposite effects is to be expected for given conditions. Here, we present the derivation of analytical expressions for the main quantifiers of transcriptional noise in terms of the molecular parameters and elucidate the role of DNA looping [1]. Our results rationalize paradoxical experimental observations and provide the first quantitative explanation of landmark individual-cell measurements at the single molecule level on the classical lac operon genetic system [2].
[1] Vilar J.M.G. and Saiz L., Physical Review E 89, (6) 062703 (2014).
[2] Choi et al., Science 322, 442-446 (2008).

2716-Pos Board B146
Nuclear Actin Dynamics Regulate Nuclear Organization and Transcription
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Actin is an important and elegant mechanotransducer that transfers messages from the extracellular environment into the nucleus, thus conferring changes in both the physical properties and the genetic program of the cell. Although actin in the nucleus has been established in a growing number of functions, the form of nuclear actin remains poorly understood as there are no apparent actin filaments in the nucleus. Therefore, we tested how disrupting the form of nuclear actin impacts function and what effects this may have on the nucleus. To do so, we formed stable nuclear actin filaments using a variety of methods, including the nuclear enrichment of actin binding proteins supervillin and α-E-catenin and the expression of a mutant form of skeletal α-actin. Using fluorescence recovery after photobleaching (FRAP), we found that stabilization of nuclear actin filaments significantly impairs actin dynamics within the nucleus. The formation of nuclear actin filaments coincides with striking changes in nuclear structure and overall nuclear topography as determined by confocal microscopy and raster image correlation spectroscopy. Using a combination of FRAP analysis, structured illumination microscopy and immunological assays, we found that nuclear actin filaments reduce the association of actin with RNA polymerase II and this correlates with impaired RNA polymerase II dynamics, localization and gene recruitment. Moreover, we were able to recapitulate our findings in purified nuclear extract by using in vitro transcription assays with the covalent actin crosslinking domain (ACD) of V. cholerae MARTX toxin. Based on our data, which help explain the absence of nuclear actin filaments in the interphase mammalian nucleus, we propose a model where nuclear actin dynamics are critical for maintaining proper nuclear function.

Chromatin and the Nucleoid

2717-Pos Board B147
Epigenetics: How Much Physics Do We Need to Understand It?
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Eukaryotic genomes are divided into chromosomes, each consisting of a single molecule of several centimeters of DNA compacted into a nucleoprotein

substance known as ''chromatin''. In the recent years, more and more evidence has accumulated pointing out chromatin polymorphism and dynamics as a primary mean of control of genome accessibility in time and space, driving the focus on this complex polymer as a critical player in gene regulation. A thorough characterization of chromatin properties would then be a prerequisite step in our understanding of differential gene expression, e.g. ''epigenetics'' in its original definition by Waddington as ''the study of the causal mechanisms by which the genes of the genotypes bring about phenotypic effects''.
We wish here to emphasize some physical characteristics of genome organization in order to provide a more complete framework in which to interpret the control of gene expression. Indeed, as various molecular motors push, pull and twist DNA, transient forces and torques develop within chromatin, with expected consequences on transcription and other DNA metabolism events such as repair or recombination. In addition to discussing some basic mechanical and topological issues, we will also present some recent quantitative and qualitative insights from our lab into chromatin organization and dynamics, including the still controversial role of ions in DNA compaction and the mechanical action of recombinases.
Boulé JB, Mozziconacci J and Lavelle C. (2014). The polymorphism of the chromatin fiber. J Phys Cond Mat (in press).
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2718-Pos Board B148
Nucleosome Kinetics and Accesibility of DNA
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Crucial cellular processes like gene regulation, transcription, and replication require access to DNA that is covered with nucleosomes. Many experiments suggest that nucleosome organization and dynamics can significantly influence exposure and accessibility of various locations on the genome. In this work we investigate the kinetics of DNA exposure as a result of nucleosome dynamics. We consider binding and dissociation of nucleosomes taking into account both sequence specificity and ATP-dependent activity, and study accessibility of DNA near different kinds of barriers (e.g. a well-positioned protein or a nucleosome free region near transcription start site). Using analytical calculations and numerical simulations, we find the following results. We show that the timescale of exposure of a DNA site near a barrier can be very diverse and crucially depends on the DNA sequence and the initial nucleosome organization. We show how nucleosome-mediated cooperativity can emerge when multiple transcription factors are binding at nearby locations and we investigate how multi-nucleosome correlations influence the time scale of accessibility as a function of the distance from the barrier. We discuss ramifications of our findings in understanding gene regulation and stochasticity in gene expression.

2719-Pos Board B149
Chromosome-Nuclear Envelope Interactions Have Multiple Effects on Chromosome Folding Dynamics in Simulation
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It is well recognized that the chromosomes of eukaryotes fold into non-random configurations within the nucleus. In humans and fruit flies, chromosomes likely adopt a particular 3D configuration called the fractal globule (FG) which has multiple biologically significant properties. However, the fractal globule is a metastable state which, over time, transitions to a less biologically favorable state called the equilibrium globule. One of the key questions is how the FG state is stabilized in-vivo? We use simulations to study the effects of chromosome-nuclear envelope (Chr-NE) interactions on the dynamics of the fractal globule within a model of Drosophila melanogaster (fruit fly) interphase chromosomes. The computational model represents chromosomes as self-avoiding walks (SAW) bounded by the nuclear envelope (NE). Model parameters such as nucleus size, chromosome persistence length, and chromosome-nuclear envelope interactions are taken directly from experiment. Several key characteristics of the non-equilibrium FG state are monitored during each simulation's progress: chromosome territories, intra-chromosomal interaction probabilities, loci specific diffusion constants, and presence of the Rabl (polarized) chromosome arrangement. Next, we compare the outcomes of simulations which include or exclude Chr-NE interactions. We find that Chr-NE interactions reinforce the non-equilibrium properties such as chromosome territories, high intra-chromosome interaction probabilities, and the Rabl chromosome arrangement. In addition, Chr-NE interactions affect loci specific and averaged chromosomal diffusion. Based on these results we conclude that the presence of Chr-NE interactions may delay the decay of the biologically relevant fractal globule state in vivo.

2720-Pos Board B150
Biophysical Models of Nucleosome Positioning
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A human body contains enough DNA to circle the Earth's Equator more than 2.5 million times. The basic units of DNA packaging are called nucleosomes. Their locations along the chromosomes play an essential role in gene regulation. We study nucleosome positioning in yeast, fly and mouse, and build biophysical models in order to explain the genome-wide nucleosome organization. We show that DNA sequence is not the major cause of the regular arrays of nucleosomes observed in vivo near the transcription start sites (TSS). We construct a minimal model in which nucleosomes are positioned by potential barriers located in the gene promoters, and which accurately reproduces the genome-wide nucleosome occupancy patterns observed over the transcribed regions in living cells. Our statistical mechanics model allows us to study nucleosome phasing against potential barriers and wells [1, 2], sequence-dependent nucleosome affinity [2], nucleosome unwrapping [3], competition between different DNA-binding proteins, and accessibility of transcription factors [4, 5] to target sites which are found in nucleosomal DNA, among others. We also discuss alternative nucleosome positioning mechanisms: nucleosome anchoring [6] and active nucleosome positioning by ATP-dependent remodelers [7].
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[5] N Elfving*, RV Chereji*, V Bharatula, S Björklund, AV Morozov, JR Broach, Nucleic Acids Res. 42, 5468 (2014) (* contributed equally)
[6] RV Chereji, AV Morozov, YM Moshkin, in preparation
[7] D Ganguli*, RV Chereji*, J Iben, HA Cole, DJ Clark, Genome Res. (2014) (* contributed equally)

2721-Pos Board B151
Prediction of Chromosome Conformations with Maximum Entropy Principle
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The genomes' three-dimensional (3D) organization is crucial in regulating many biological processes, including gene regulation, DNA replication, and cell differentiation. A high-resolution chromosome structure thus will significantly advance our understanding of these important processes. A major step toward building a structural model of the chromosome is the inventions of chromosome conformation capture methods, 5C and Hi-C, that aim at detecting physical contact frequencies between pairs of genomic loci. However, computational approaches to construct 3D structures that are consistent with these experimental contact frequency measurements remain lacking.
We develop a statistically rigorous approach based on maximum entropy principle to determine a least-biased potential energy landscape that reproduces experimentally determined Hi-C contact frequency between genome pairs. The resulting energy landscape supports a knotless chromosome conformation, which has been highly anticipated since complex knotted conformations prohibit the access of gene information for transcription and hinder DNA replication. We further show that the topologically associating domain signal alone also enforces a chromosome structure free of knots. Our results highlight the importance of local interactions in determining the global topology of the chromosome structure. Finally, the derived landscapes for multiple chromosomes support the formation of territories that have long been observed in microscopy experiments. Together with Hi-C experiments, our approach provides a coherent picture of the 3D architecture of the genomes that is consistent with many the available experimental data.

2722-Pos Board B152
Modeling the Binding of H-NS to DNA
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Bacterial chromosomal DNA is organized within a structure called the nucleoid, which is distinctly different from the rest of the cytoplasm. Bacteria have a number of nucleoid-associated proteins that influence the organization of the